White Paper

INGOLD
Leading Process Analytics

# 5 Ways to Avoid Deviations
## from Process to Lab

**Achieving reproducible pH measurements from the lab to the production environment can be very challenging. A few tips can easily help you stay in control.**

pH is a critical parameter in development and scale-up of cell culture and microbial bioprocesses, as well as in most downstream applications. Bioreactors, even the smallest, are equipped with pH sensors for process control, but often deviations between in-line and off-line sample measurements can reach up to a whole pH unit, creating uncertainty and additional workload. Here are 5 tips for minimizing error sources and regaining process control.

**1. Compare measurements at the same temperature**

Modern pH probes come with built-in temperature sensors and automatically correct the slope for temperature changes, but this is not enough to avoid all errors related to temperature differences.

Chemical equilibria are temperature dependent; therefore, the same sample will have a different pH at different temperatures. Compensating for this error is only possible when certain media characteristics are known. These are typically only available for pH buffer solutions.

To avoid this error, make sure you compare measurements taken at the same temperature.

**2. Select the right pH sensor**

Culture media, as well as other typical process media, have heterogeneous and complex chemical compositions that interact with the sensor reference system. These interactions are sources of error for pH measurement. They can be minimized by selecting the right sensor type, but can never be entirely eliminated.

For bioprocesses, sensors with a pre-pressurized liquid electrolyte reference system are typically the best choice, because they show the highest reproducibility through several sterilization cycles.

METTLER TOLEDO

Comparing results from similar sensor types helps minimize measurement errors. Choose similar pH sensor types for in-line as well as laboratory measurements.

**3. Recalibrate after sterilization**

In-line sensors are commonly sterilized inside the bioreactor. The high temperature of SIP procedures affect pH sensor membrane glass performance. Glass formulations that are especially developed to withstand high temperatures will minimize these problems.

Nevertheless, when necessary, it is possible to perform a so-called 'process calibration'. It consists of a one-point calibration where the pH in-line reading is standardized to a sample off-line measurement.

Before a process calibration, always make sure the sensor has cooled down to process temperature and has reached a stable signal. Also, be aware that during certain process phases, pH can change very quickly in the process medium. To avoid errors, most transmitters offer a dedicated function for process calibrations. In a first step, the pH reading at the moment the sample is taken is stored in the transmitter's memory.

The off-line sample can be measured in the lab and, in a second step, entered into the transmitter to adjust the sensor. This procedure allows accurate calibration even if the process conditions change.

In general, it is best to measure the off-line sample as quickly as possible in a nearby lab, or use a portable pH meter if the laboratory is too far away. When this is not possible, rapidly cooling the sample to 4 °C and storing it in a closed container is the best way to minimize temperature-related errors.

Viscous or protein containing samples can clog a pH sensor's junction and interfere with measurement performance. Use maintenance solutions such as Thiourea or Pepsin-HCl to clean junctions to ensure accuracy and measurement precision of laboratory and in-line sensors.

Process calibration

**4. Standardize the sampling procedure**

With so many variables influencing pH measurement, the best way to reduce result variability between different operators, is to standardize sampling procedures.

The sample should be representative of the process conditions at the moment it is taken. In small bioreactors this means making sure the sampling tube is completely empty before taking a fresh sample. In large-scale bioreactors it will be necessary to drain the liquid for one or two minutes after opening the sample valve.

The sampling procedure should describe not only when and how the sample is taken, but also for how long and how the sample should be stored before measurement. At higher viscosities, sample mixing will also play a major role.

pH calibration in the lab

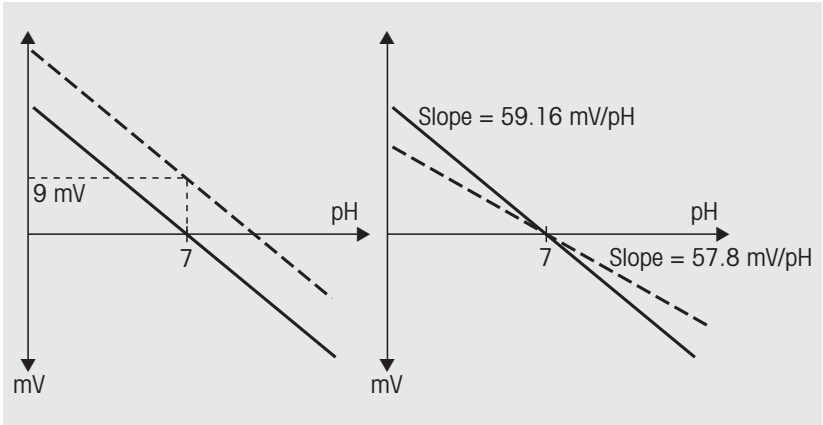
**5. Standardize the measurement method**

A laboratory pH sensor needs to be calibrated regularly, at least on the same day the measurement is performed. The calibration adjusts the slope and offset of the sensor to the true measured values of defined pH standards. For a wide sample pH range, a three-point calibration, e.g. pH 4.01, 7.00 and 9.21, is usually recommended. Choose the calibration solutions such that the expected pH of the material under test falls within their range. Maintain the same temperature during the calibration of the sensor and the following measurement. Due to the dependence of the pH measurement on the calibration, ensure that calibration solutions are of high quality, always fresh, and not expired.

In laboratory pH meters, the user has the option between different endpoint criteria. Too fast a measurement could lead to measurement deviation with an in-line sensor, which measures continuously and is in equilibrium with the media. To reduce deviations in the pH measurement of the samples, adjust the laboratory measurement settings of the meter allowing enough time for the measurement to stabilize.

Following these steps will ensure your pH measurements are always accurate and reliable.

**www.mt.com/pH-Sensor**




Adjust the offset and slope

## Recommended METTLER TOLEDO pH sensors for bioprocesses

### InPro 3253i*

Ensures batch-to-batch consistency thanks to pre-pressurized liquid electrolyte and steam sterilizable glass.

| | |
|---|---|
| pH range | 0 – 12 pH |
| Reference system | Argenthal™ with silver-ion trap (compatible with TRIS buffer) |
| Type of junction | Ceramic junction |
| Reference electrolyte | Pre-pressurized liquid |
| pH membrane | Steam sterilizable glass |

* also available with high alkali glass and extended pH range 0 – 14 pH

### InLab® Power Pro-ISM (Mat. n° 51344211)

Designed for general laboratory applications; highly reproducible results even in challenging samples containing polymeric dispersions.

| | |
|---|---|
| pH range | 0 – 12 pH |
| Reference system | Argenthal with silver-ion trap (compatible with TRIS buffer) |
| Type of junction | Ceramic junction |
| Reference electrolyte | Pre-pressurized gel |
| pH membrane | Steam sterilizable glass |

### InPro 3100i

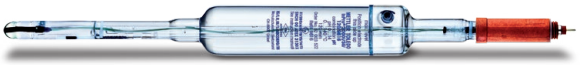
This sterilizable electrode combines a robust and precise gel-filled reference system with a versatile design that allows for upside-down mounting (InPro® 3100i UD).

| | |
|---|---|
| pH range | 0 – 14 pH |
| Reference system | Argenthal with silver-ion trap (compatible with TRIS buffer) |
| Type of junction | Ceramic junction |
| Reference electrolyte | Gel |
| pH membrane | High alkali glass |

### InLab Viscous Pro-ISM (Mat. n° 51343151)

A probe designed for viscous samples and well suited for protein containing media.

| | |
|---|---|
| pH range | 0 – 14 pH |
| Reference system | Argenthal with silver-ion trap (compatible with TRIS buffer) |
| Type of junction | Ceramic junction |
| Reference electrolyte | Pre-pressurized gel |
| pH membrane | High alkali glass |



### InPro 2000i

Thanks to the refillable reference system, this sensor has a long lifetime. Available with different liquid electrolytes, it can be pressurized in the housing for accurate results even with the most extreme requirements.

| | |
|---|---|
| pH range | 0 – 14 pH |
| Reference system | Argenthal with silver-ion trap (compatible with TRIS buffer |
| Type of junction | Ceramic junction |
| Reference electrolyte | 3M KCl, Viscolyt™ or Friscolyt |
| pH membrane | High alkali glass |

### InLab Routine Pro-ISM (Mat. n° 51344055)

A glass body combination pH electrode with integrated temperature sensor and Intelligent Sensor Management (ISM®) technology. Designed for general laboratory applications; fast and highly chemical resistant.

| | |
|---|---|
| pH range | 0 – 14 pH |
| Reference system | Argenthal with silver-ion trap (compatible with TRIS buffer) |
| Type of junction | Ceramic junction |
| Reference electrolyte | 3M KCl |
| pH membrane | High alkali glass |



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